

Supplementary Material

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Article: Tramps in transition: genetic differentiation between populations of an iconic "supertramp" taxon in the Central Indo-Pacific

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Summary:

Figure S1. Example PCR protocol used in this study

Figure S2. Diagram of the mitochondrial ND2 gene showing how we used internal primers to sequence the gene in two halves.

Figure S3. Full Bayesian tree showing all haplotypes.

Figure S4. Full Maximum Likelihood (ML) tree showing all haplotypes.

Table S1. DNA samples used in this study.

Table S2. PCR primers used in this study.

Table S3. Proportion difference (p-distance) between concatenated ND2-ND3 haplotypes in *Hypothymis*

Table S4. P-distances between concatenated ND2-ND3 haplotypes in *Monarcha*

Appendix S1. Expanded "Genetic Analyses" Section, with more detail on phylogenetic modelling, and R code used in analyses

Supplementary Figures

P449		ND2			01/10/2020	Machine:	BioMetra T Pro			
				16	aliquots		lid temp 97°C			
MASTER MIX RECIPE							Phase 1			
193.6	μl		12.1	μl	ddH2O		95°	for	180 s	
9.6	μl		0.6	μl	dNTPs (10 mM each)		Touch Down: 15 cycles of			
48	μl		3.0	μl	10x buffer		95°	for	30 s	
57.6	μl		3.6	μl	MgCl2 (25 mM)		62°	for	45 s	ΔTa -1°
24	μl		1.5	μl	forward primer (10 μM)	MonND2intF1	72°	for	60 s	
24	μl		1.5	μl	reverse primer (10 μM)	FOM-WANCY-R2	Phase 2: 20 cycles of			
3.2	μl		0.2	μl	Taq (Invitrogen)		95°	for	30 s	
360							47°	for	45 s	
			22.5	μl	Master Mix		72°	for	60 s	
			7.5	μl	DNA - more template		Termination			
			30.0	μl	Total volume for screen and sequence		72°	for	300 s	
							4°	for	ever	
						protocol name:	ND2 62 TDN			

Figure S1. Example PCR protocol used in this study, showing the reagents and quantities in the master mix along with the thermal cycling program used. We used “touchdown” cycling programs to improve yield (Korbie and Mattick 2008), starting at an annealing temperature (62°C in this example) that’s 10° above the melting temperature of the primers. The annealing temperature then drops (or “touches down”) by 1° per cycle over the following 14 cycles.

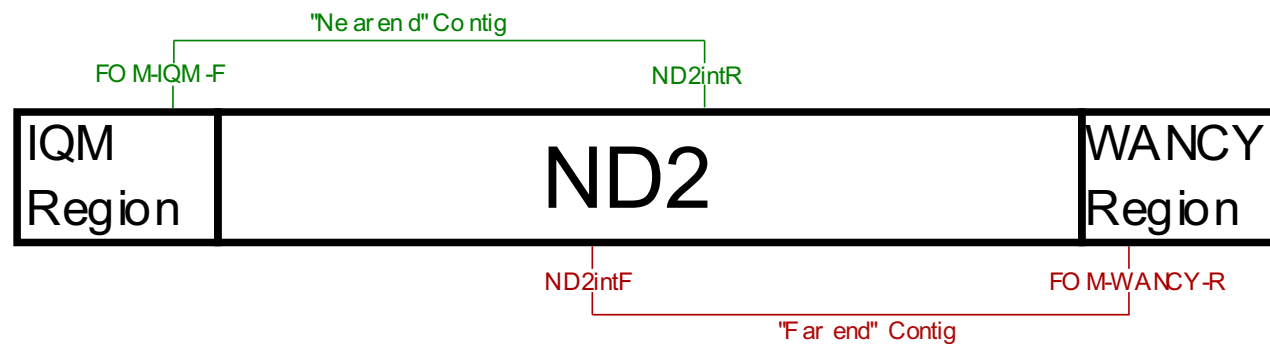


Figure S2. Diagram of the mitochondrial ND2 gene showing how we used internal primers to sequence the gene in two halves. We designed primers to bind to the IQM and WANCY regions and two sites in the ND2 gene, to give two contiguous gene reads (contigs) with an area of overlap to allow them to be aligned. More information on our primers is available in Table S2.

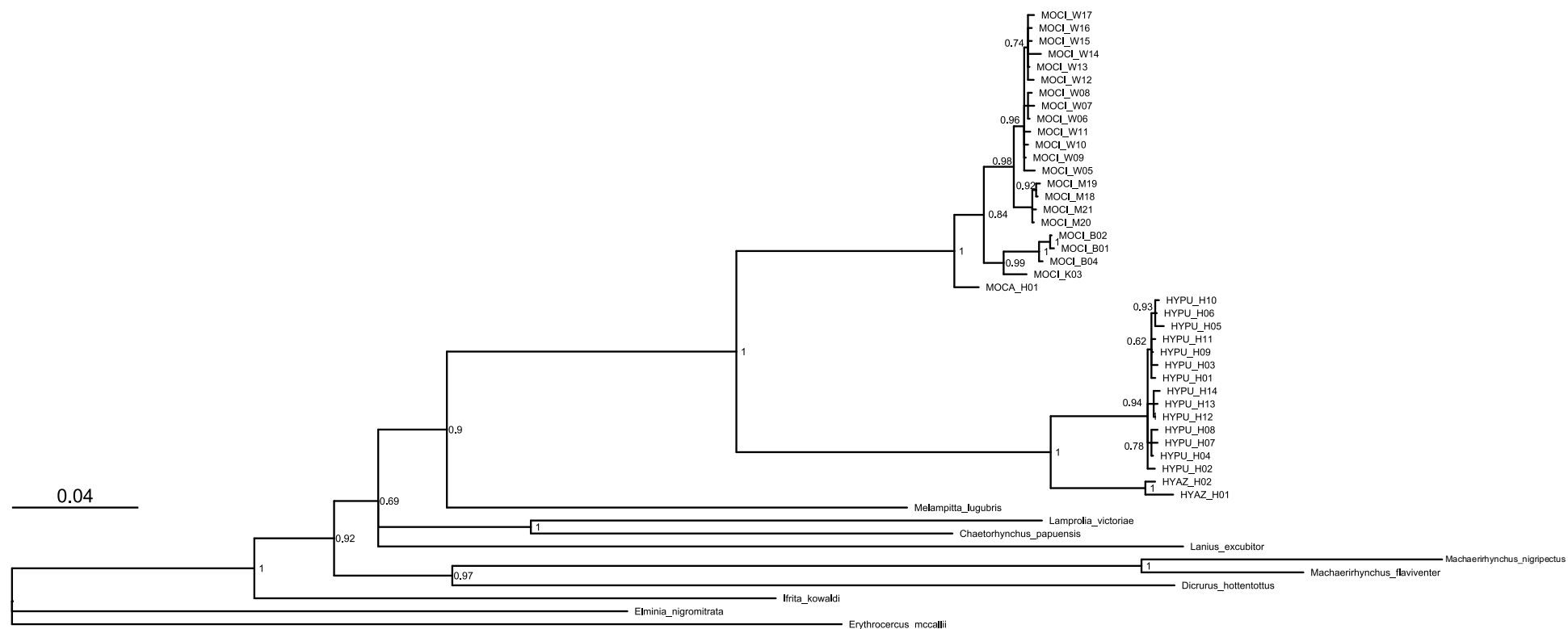


Figure S3. Full Bayesian tree showing all haplotypes. Nodes are labelled with Bayesian probabilities.

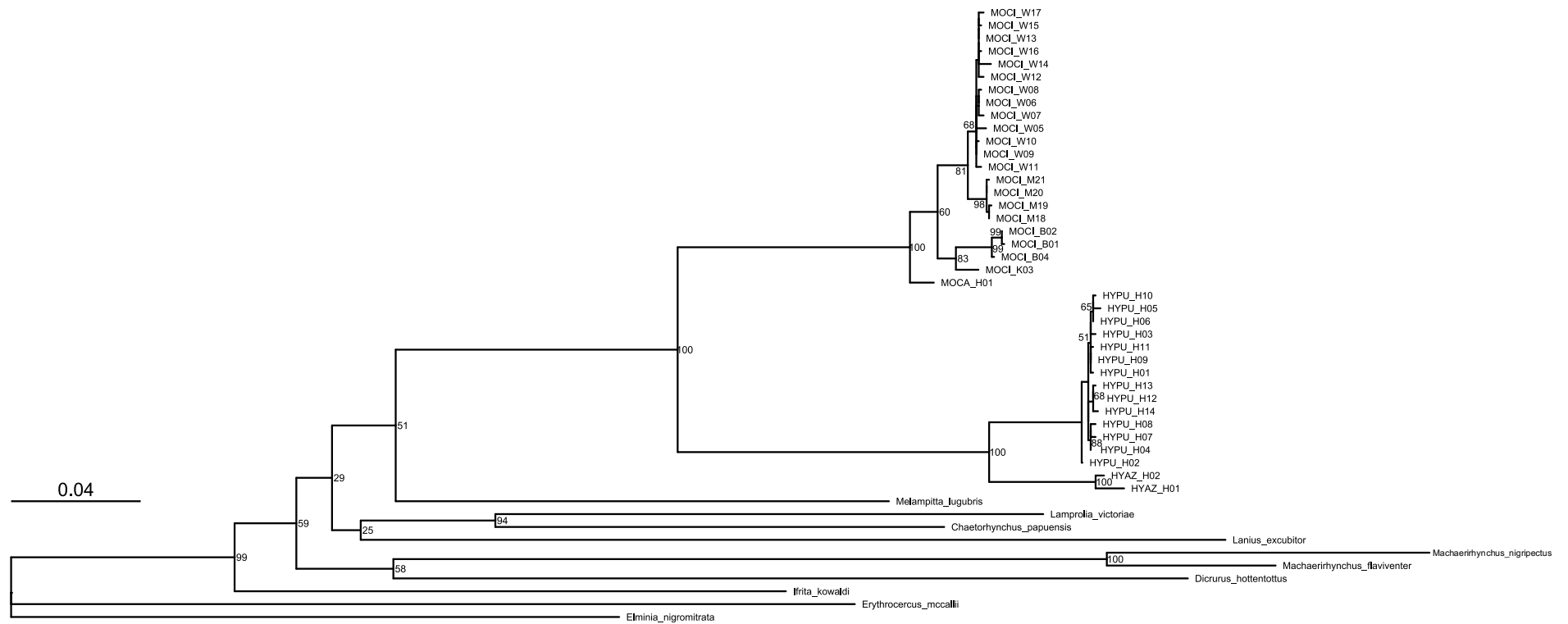


Figure S4. Full Maximum Likelihood (ML) tree showing all haplotypes. Nodes are labelled with bootstrap values.

Supplementary Tables

Table S1. DNA samples used in this study.

Species	ID	Concatenated ND2-ND3 haplotype	ND2 Accession	ND3 Accession	Locality	Latitude	Longitude	Source
<i>Hypothymis azurea</i>	KUNHM 10293	HYAZ_H01	KP069724	KP069815	Guangxi Region, China	21.86	107.84	Andersen et al. (2015)
<i>Hypothymis azurea</i>	KUNHM 13983	HYAZ_H02	KP069627	KP069810	Camiguin Sur, Philippines	9.1925167	124.70845	Andersen et al. (2015)
<i>Hypothymis puella</i>	SUL0565	HYPH_H01	MZ604441	MZ604497	Enano, Kabaena Island, Indonesia	-5.247466667	121.9567	This study
<i>Hypothymis puella</i>	SUL0600	HYPH_H02	MZ604442	MZ604498	Enano, Kabaena Island, Indonesia	-5.26493	121.9696	This study
<i>Hypothymis puella</i>	SUL0641	HYPH_H03	MZ604443	MZ604499	Enano, Kabaena Island, Indonesia	-5.26493	121.9696	This study
<i>Hypothymis puella</i>	SUL1332	HYPH_H04	MZ604444	MZ604500	Rumbarumba, Southeast Sulawesi, Indonesia	-4.42	122.804	This study
<i>Hypothymis puella</i>	SUL1335	HYPH_H05	MZ604445	MZ604501	Rumbarumba, Southeast Sulawesi, Indonesia	-4.42	122.804	This study
<i>Hypothymis puella</i>	SUL1336	HYPH_H06	MZ604446	MZ604502	Rumbarumba, Southeast Sulawesi, Indonesia	-4.42	122.804	This study
<i>Hypothymis puella</i>	SUL1916	HYPH_H07	MZ604447	MZ604503	Waode Buri, Buton Island, Indonesia	-4.69027	123.20753	This study
<i>Hypothymis puella</i>	SUL1917	HYPH_H08	MZ604448	MZ604504	Waode Buri, Buton Island, Indonesia	-4.69027	123.20753	This study
<i>Hypothymis puella</i>	SUL2046	HYPH_H09	MZ604449	MZ604505	Universitas Halu Oleo, Southeast Sulawesi, Indonesia	-4.0136	122.5255	This study
<i>Hypothymis puella</i>	SUL2059	HYPH_H10	MZ604450	MZ604506	Universitas Halu Oleo, Southeast Sulawesi, Indonesia	-4.0136	122.5255	This study
<i>Hypothymis puella</i>	SUL2061	HYPH_H11	MZ604451	MZ604507	Universitas Halu Oleo, Southeast Sulawesi, Indonesia	-4.0136	122.5255	This study
<i>Hypothymis puella</i>	SUL2287	HYPH_H12	MZ604452	MZ604508	Kamama Mekar, Muna Island, Indonesia	-5.31328	122.6421	This study

<i>Hypothymis puella</i>	SUL2288	HYPU_H13	MZ604453	MZ604509	Kamama Mekar, Muna Island, Indonesia	-5.31328	122.6421	This study
<i>Hypothymis puella</i>	SUL2306	HYPU_H14	MZ604454	MZ604510	Kamama Mekar, Muna Island, Indonesia	-5.30763	122.63847	This study
<i>Monarcha castaneiventris</i>	KUNHM 15914	MOCA_H01	GQ145385	GQ145423	Makira Island, Solomon Islands	-10.4585	161.943	Nyári et al. (2009)
<i>Monarcha cinerascens perpallidus</i>	KUNHM 27791	MOCI_B01	KP069680	KP069913	Nusalaman Island, Bismarck Archipelago, Papua New Guinea	not provided	not provided	Andersen et al. (2015)
<i>Monarcha cinerascens perpallidus</i>	KUNHM 27858	MOCI_B02	KP069587	KP069761	Djaul Island, Bismarck Archipelago, Papua New Guinea	not provided	not provided	Andersen et al. (2015)
<i>Monarcha cinerascens nigrirostris</i>	KUNHM 27870	MOCI_K03	KP069753	KP069901	Karkar Island, Bismarck Archipelago, Papua New Guinea	-4.6617833	145.9690333	Andersen et al. (2015)
<i>Monarcha cinerascens impediens</i>	UWBM 60337	MOCI_B04	KP069703	KP069835	Sulei Island, Solomon Islands	-8.08	159.5368	Andersen et al. (2015)
<i>Monarcha cinerascens</i>	SUL0983	MOCI_W05	MZ604455	MZ604511	Rukuwa, Binongko Island, Indonesia	-5.92005	124.04613	This study
<i>Monarcha cinerascens</i>	SUL0986	MOCI_W05	MZ604456	MZ604512	Rukuwa, Binongko Island, Indonesia	-5.92005	124.04613	This study
<i>Monarcha cinerascens</i>	SUL0996	MOCI_W05	MZ604457	MZ604513	Rukuwa, Binongko Island, Indonesia	-5.92005	124.04613	This study
<i>Monarcha cinerascens</i>	SUL0998	MOCI_W05	MZ604458	MZ604514	Rukuwa, Binongko Island, Indonesia	-5.92005	124.04613	This study
<i>Monarcha cinerascens</i>	SUL1048	MOCI_W05	MZ604459	MZ604515	Rukuwa, Binongko Island, Indonesia	-5.89817	124.0223	This study
<i>Monarcha cinerascens</i>	SUL1066	MOCI_W06	MZ604460	MZ604516	Runduma Island, Indonesia	-5.3336	124.3363	This study
<i>Monarcha cinerascens</i>	SUL1091	MOCI_W07	MZ604461	MZ604517	Runduma Island, Indonesia	-5.3336	124.3363	This study
<i>Monarcha cinerascens</i>	SUL1093	MOCI_W06	MZ604462	MZ604518	Runduma Island, Indonesia	-5.3336	124.3363	This study
<i>Monarcha cinerascens</i>	SUL1102	MOCI_W08	MZ604463	MZ604519	Runduma Island, Indonesia	-5.3336	124.3363	This study
<i>Monarcha cinerascens</i>	SUL1144	MOCI_W09	MZ604464	MZ604520	Lintea Selatan Island, Indonesia	-5.803	123.9057	This study
<i>Monarcha cinerascens</i>	SUL1191	MOCI_W10	MZ604465	MZ604521	Lintea Selatan Island, Indonesia	-5.80282	123.90405	This study

<i>Monarcha cinerascens</i>	SUL1539	MOCI_W11	MZ604466	MZ604522	Melai One, Wangi-wangi Island, Indonesia	-5.34511	123.55353	This study
<i>Monarcha cinerascens</i>	SUL1595	MOCI_W12	MZ604467	MZ604523	Air Nounou, Kaledupa Island, Indonesia	-5.50895	123.7501	This study
<i>Monarcha cinerascens</i>	SUL1596	MOCI_W13	MZ604468	MZ604524	Air Nounou, Kaledupa Island, Indonesia	-5.50895	123.7501	This study
<i>Monarcha cinerascens</i>	SUL1616	MOCI_W14	MZ604469	MZ604525	Latafe, Kaledupa Island, Indonesia	-5.51379	123.74953	This study
<i>Monarcha cinerascens</i>	SUL1617	MOCI_W12	MZ604470	MZ604526	Latafe, Kaledupa Island, Indonesia	-5.51379	123.74953	This study
<i>Monarcha cinerascens</i>	SUL1619	MOCI_W15	MZ604471	MZ604527	Latafe, Kaledupa Island, Indonesia	-5.51379	123.74953	This study
<i>Monarcha cinerascens</i>	SUL1622	MOCI_W09	MZ604472	MZ604528	Latafe, Kaledupa Island, Indonesia	-5.51379	123.74953	This study
<i>Monarcha cinerascens</i>	SUL1654	MOCI_W13	MZ604473	MZ604529	Hoga Island, Indonesia	-5.46474	123.76133	This study
<i>Monarcha cinerascens</i>	SUL1655	MOCI_W13	MZ604474	MZ604530	Hoga Island, Indonesia	-5.46474	123.76133	This study
<i>Monarcha cinerascens</i>	SUL1711	MOCI_W16	MZ604475	MZ604531	Fatu Kokahu, Tomia Island, Indonesia	-5.72707	123.90618	This study
<i>Monarcha cinerascens</i>	SUL1712	MOCI_W09	MZ604476	MZ604532	Fatu Kokahu, Tomia Island, Indonesia	-5.72707	123.90618	This study
<i>Monarcha cinerascens</i>	SUL1714	MOCI_W09	MZ604477	MZ604533	Fatu Kokahu, Tomia Island, Indonesia	-5.72707	123.90618	This study
<i>Monarcha cinerascens</i>	SUL1718	MOCI_W16	MZ604478	MZ604534	Fatu Kokahu, Tomia Island, Indonesia	-5.72707	123.90618	This study
<i>Monarcha cinerascens</i>	SUL1729	MOCI_W09	MZ604479	MZ604535	Fatu Kokahu, Tomia Island, Indonesia	-5.7344	123.9071	This study
<i>Monarcha cinerascens</i>	SUL1873	MOCI_W17	MZ604480	MZ604536	Hoga Island, Indonesia	-5.46474	123.76133	This study
<i>Monarcha cinerascens</i>	SUL1874	MOCI_W13	MZ604481	MZ604537	Hoga Island, Indonesia	-5.46474	123.76133	This study
<i>Monarcha cinerascens</i>	SUL2197	MOCI_W13	MZ604482	MZ604538	Melai One, Wangi-wangi Island, Indonesia	-5.337817	123.614767	This study
<i>Monarcha cinerascens</i>	SUL2202	MOCI_W13	MZ604483	MZ604539	Melai One, Wangi-wangi Island, Indonesia	-5.337817	123.614767	This study
<i>Monarcha cinerascens</i>	SUL2210	MOCI_W13	MZ604484	MZ604540	Melai One, Wangi-wangi Island, Indonesia	-5.337817	123.614767	This study
<i>Monarcha cinerascens</i>	SUL2215	MOCI_W09	MZ604485	MZ604541	Melai One, Wangi-wangi Island, Indonesia	-5.337817	123.614767	This study
<i>Monarcha cinerascens</i>	SUL2216	MOCI_W09	MZ604486	MZ604542	Melai One, Wangi-wangi Island, Indonesia	-5.337817	123.614767	This study
<i>Monarcha cinerascens</i>	SUL2219	MOCI_W09	MZ604487	MZ604543	Melai One, Wangi-wangi Island, Indonesia	-5.337817	123.614767	This study

<i>Monarcha cinerascens</i>	SUL2472	MOCI_M18	MZ604488	MZ604544	Buranga, Menui Island, Indonesia	-3.584	123.1199	This study
<i>Monarcha cinerascens</i>	SUL2504	MOCI_M19	MZ604489	MZ604545	Buranga, Menui Island, Indonesia	-3.5751	123.0832	This study
<i>Monarcha cinerascens</i>	SUL2514	MOCI_M20	MZ604490	MZ604546	Buranga, Menui Island, Indonesia	-3.5732	123.1405	This study
<i>Monarcha cinerascens</i>	SUL2541	MOCI_M18	MZ604491	MZ604547	Buranga, Menui Island, Indonesia	-3.5739	123.09105	This study
<i>Monarcha cinerascens</i>	SUL2542	MOCI_M18	MZ604492	MZ604548	Buranga, Menui Island, Indonesia	-3.5739	123.09105	This study
<i>Monarcha cinerascens</i>	SUL2547	MOCI_W09	MZ604493	MZ604549	Buranga, Menui Island, Indonesia	-3.5773	123.0791	This study
<i>Monarcha cinerascens</i>	SUL2548	MOCI_M20	MZ604494	MZ604550	Buranga, Menui Island, Indonesia	-3.5773	123.0791	This study
<i>Monarcha cinerascens</i>	SUL2576	MOCI_M20	MZ604495	MZ604551	Buranga, Menui Island, Indonesia	-3.5767	123.0751	This study
<i>Monarcha cinerascens</i>	SUL2581	MOCI_M21	MZ604496	MZ604552	Buranga, Menui Island, Indonesia	-3.5766	123.0751	This study
<i>Chaetorhynchus papuensis</i>	KUNHM 6974	OUTGROUP	KP069618	KP069869	Oro Province, Papua New Guinea	-9.5264333	149.10465	Andersen et al. (2015)
<i>Dicrurus hottentottus</i>	KUNHM 10036	OUTGROUP	KP069686	KP069805	Guangxi, China	not provided	not provided	Andersen et al. (2015)
<i>Elminia nigromitratus</i>	FMNH 391789	OUTGROUP	KP069752	KP069836	Western Region, Uganda	1.70889	31.5261	Andersen et al. (2015)
<i>Erythrocerus mcallii</i>	FMNH 396684	OUTGROUP	KP069609	KP069846	Central Region, Ghana	5.33333	-1.21667	Andersen et al. (2015)
<i>Ifrita kowaldi</i>	KUNHM 16238	OUTGROUP	KP069628	KP069843	Central Province, Papua New Guinea	-9.9998167	149.5071833	Andersen et al. (2015)
<i>Lamprolia victoriae</i>	KUNHM 24276	OUTGROUP	KP069621	KP069803	Vanua Levu Island, Fiji	-16.6366667	179.7616667	Andersen et al. (2015)
<i>Lanius excubitor</i>	KUNHM 28984	OUTGROUP	KP069730	KP069799	Gobi Altai, Mongolia	45.538	93.651	Andersen et al. (2015)
<i>Machaerirhynchus flaviventer</i>	KUNHM 5139	OUTGROUP	DQ084072	KP069804	Simbu Province, Papua New Guinea	-6.7885	145.03675	Andersen et al. (2015)
<i>Machaerirhynchus nigripectus</i>	KUNHM 4734	OUTGROUP	KP069750	KP069828	Morobe Province, Papua New Guinea	-6.08165	146.57225	Andersen et al. (2015)
<i>Melampitta lugubris</i>	KUNHM 16522	OUTGROUP	KP069651	KP069917	Eastern Highlands Province, Papua New Guinea	-7.0610667	145.8244	Andersen et al. (2015)

Table S2. PCR primers used in this study.

Primer Name	Locus	Description	Direction	Sequence (5' to 3')	Source
L10755-F	ND3	External, general	Forward	GACTTCCAATCTTTAAAATCTGG	Chesser (1999)
DOC-ND3-R1	ND3	External, general	Reverse	TTGTTGAGTCGAAATCAACTG	O'Connell et al. (2019)
FOM-IQM-F1	ND2	External, general	Forward	ATACCCCGRAAATGATGG	This study
FOM-WANCY-R2	ND2	External, general	Reverse	TGTTTAAGGCTTTGAAGGC	Ó Marcaigh et al. (2021)
HypND2intR1	ND2	Internal for <i>Hypothymis</i>	Reverse	GAATAATGAGTCACTTCGG	This study
HypND2intF1	ND2	Internal for <i>Hypothymis</i>	Forward	TAGGCTTAGTACCATTCC	This study
MonND2intR1	ND2	Internal for <i>Monarcha</i>	Reverse	GGAGGAATCCTGTTAAGG	This study
MonND2intF1	ND2	Internal for <i>Monarcha</i>	Forward	TAGGCCTAGTACCATTCC	This study

Table S3. Proportion difference (p-distance) between concatenated ND2-ND3 haplotypes in *Hypothymis*

	HYAZ_ H01	HYAZ_ H02	HYPH_ H01	HYPH_ H02	HYPH_ H03	HYPH_ H04	HYPH_ H05	HYPH_ H06	HYPH_ H07	HYPH_ H08	HYPH_ H09	HYPH_ H10	HYPH_ H11	HYPH_ H12	HYPH_ H13	HYPH_ H14
HYAZ_ H01																
HYAZ_ H02	0.0108															
HYPH_ H01	0.0596	0.0546														
HYPH_ H02	0.0589	0.0539	0.0036													
HYPH_ H03	0.0603	0.0553	0.0022	0.0043												
HYPH_ H04	0.0589	0.0539	0.0022	0.0029	0.0029											
HYPH_ H05	0.0618	0.0568	0.0036	0.0057	0.0043	0.0043										
HYPH_ H06	0.0596	0.0546	0.0014	0.0036	0.0022	0.0022	0.0022									
HYPH_ H07	0.0603	0.0553	0.0036	0.0043	0.0043	0.0014	0.0057	0.0036								
HYPH_ H08	0.0603	0.0553	0.0036	0.0043	0.0043	0.0014	0.0057	0.0036	0.0029							
HYPH_ H09	0.0582	0.0532	0.0014	0.0036	0.0022	0.0022	0.0036	0.0014	0.0036	0.0036						
HYPH_ H10	0.0603	0.0553	0.0022	0.0043	0.0029	0.0029	0.0029	0.0007	0.0043	0.0043	0.0022					
HYPH_ H11	0.0596	0.0546	0.0014	0.0036	0.0022	0.0022	0.0036	0.0014	0.0036	0.0036	0.0014	0.0022				
HYPH_ H12	0.0596	0.0546	0.0029	0.0036	0.0036	0.0022	0.0050	0.0029	0.0036	0.0036	0.0029	0.0036	0.0029			
HYPH_ H13	0.0603	0.0553	0.0036	0.0043	0.0043	0.0029	0.0057	0.0036	0.0043	0.0043	0.0036	0.0043	0.0036	0.0007		
HYPH_ H14	0.0596	0.0546	0.0043	0.0050	0.0050	0.0036	0.0065	0.0043	0.0050	0.0050	0.0043	0.0050	0.0043	0.0014	0.0022	

Table S4. P-distances between concatenated ND2-ND3 haplotypes in *Monarcha*

	MOCA_H01	MOCI_B01	MOCI_B02	MOCI_K03	MOCI_B04	MOCI_W05	MOCI_W06	MOCI_W07	MOCI_W08	MOCI_W09	MOCI_W10	MOCI_W11	MOCI_W12	MOCI_W13	MOCI_W14	MOCI_W15	MOCI_W16	MOCI_W17	MOCI_M18	MOCI_M19	MOCI_M20
MOCI_B01	0.0287																				
MOCI_B02	0.0295	0.0007																			
MOCI_K03	0.0251	0.0201	0.0194																		
MOCI_B04	0.0273	0.0043	0.0036	0.0172																	
MOCI_W05	0.0251	0.0295	0.0287	0.0230	0.0266																
MOCI_W06	0.0259	0.0287	0.0280	0.0223	0.0259	0.0022															
MOCI_W07	0.0273	0.0287	0.0280	0.0237	0.0259	0.0036	0.0014														
MOCI_W08	0.0266	0.0295	0.0287	0.0230	0.0266	0.0029	0.0007	0.0022													
MOCI_W09	0.0251	0.0280	0.0273	0.0216	0.0251	0.0014	0.0007	0.0022	0.0014												
MOCI_W10	0.0259	0.0287	0.0280	0.0223	0.0259	0.0022	0.0014	0.0029	0.0022	0.0007											
MOCI_W11	0.0266	0.0295	0.0287	0.0230	0.0266	0.0029	0.0022	0.0036	0.0029	0.0014	0.0022										
MOCI_W12	0.0244	0.0302	0.0295	0.0237	0.0273	0.0022	0.0029	0.0043	0.0036	0.0022	0.0029	0.0036									
MOCI_W13	0.0244	0.0287	0.0280	0.0223	0.0259	0.0007	0.0014	0.0029	0.0022	0.0007	0.0014	0.0022	0.0014								
MOCI_W14	0.0280	0.0323	0.0316	0.0259	0.0295	0.0043	0.0050	0.0065	0.0057	0.0043	0.0050	0.0057	0.0050	0.0036							
MOCI_W15	0.0251	0.0295	0.0287	0.0230	0.0266	0.0000	0.0022	0.0036	0.0029	0.0014	0.0022	0.0029	0.0022	0.0007	0.0043						
MOCI_W16	0.0251	0.0295	0.0287	0.0230	0.0266	0.0014	0.0022	0.0036	0.0029	0.0014	0.0022	0.0029	0.0022	0.0007	0.0043	0.0014					
MOCI_W17	0.0259	0.0302	0.0295	0.0237	0.0273	0.0022	0.0029	0.0043	0.0036	0.0022	0.0029	0.0036	0.0029	0.0014	0.0050	0.0022	0.0022				
MOCI_M18	0.0259	0.0295	0.0287	0.0230	0.0266	0.0101	0.0093	0.0108	0.0101	0.0086	0.0093	0.0086	0.0093	0.0093	0.0129	0.0101	0.0101	0.0108			
MOCI_M19	0.0266	0.0302	0.0295	0.0237	0.0273	0.0108	0.0101	0.0115	0.0108	0.0093	0.0101	0.0093	0.0101	0.0101	0.0136	0.0108	0.0108	0.0115	0.0007		
MOCI_M20	0.0251	0.0302	0.0295	0.0237	0.0273	0.0093	0.0086	0.0101	0.0093	0.0079	0.0086	0.0079	0.0086	0.0086	0.0122	0.0093	0.0093	0.0101	0.0007	0.0014	
MOCI_M21	0.0259	0.0309	0.0302	0.0244	0.0280	0.0101	0.0093	0.0108	0.0101	0.0086	0.0093	0.0086	0.0093	0.0093	0.0129	0.0101	0.0101	0.0108	0.0014	0.0022	0.0007

Appendix S1. Expanded “Genetic Analyses” Section, with more detail on phylogenetic modelling, and R code used in analyses

Expanded “Genetic Analyses” Section

The bolded text provides additional detail over the Materials and Methods section of the main text.

We used both Maximum Likelihood (ML) and Bayesian approaches to investigate the phylogenetics of monarchs. Only one representative of each concatenated ND2/ND3 haplotype was included in the phylogenies. We used MEGA X version 10.1.8 (Kumar et al. 2018) to calculate the pairwise p-distances between these haplotypes (Tables S3 and S4). A p-distance is the proportion of sites that are different between the two sequences. We used the ModelFinder function (Kalyaanamoorthy et al. 2017) in IQTree version 1.6.12 (Nguyen et al. 2015) to select the most appropriate nucleotide substitution model for our alignment. **A nucleotide substitution model defines the probability of a given nucleotide base in the gene being replaced by another, so selecting an appropriate model is essential to calculating the likelihood of a phylogenetic tree (Sullivan and Joyce 2005, Posada and Crandall 2021).** Our ModelFinder analysis found strong support for a General Time Reversible model with a gamma shape parameter and a proportion of invariable sites (GTR+F+I+G4), supported by lower Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC) than other models. **These two criteria assess model performance differently: a lower AIC indicates that less information is being “lost” by the model, while BIC measures “lack of fit” (Johnson and Omland 2004).** The model selected (GTR+F+I+G4) implies that mutations in these genes can occur and subsequently reverse as time passes (Lanave et al. 1984, Tavaré 1986), but that some sites in the gene do not change due to biological constraints (Waddell and Steel 1997).

We conducted our ML analysis in IQTree version 1.6.12 (Nguyen et al. 2015) using a GTR+F+I+G4 model. The confidence interval of the ML phylogeny was based on 1000 bootstraps, **resampling points from our dataset with replacement and counting how many of these bootstrap trees supported each monophyletic grouping** (Felsenstein 1985). We used MrBayes version 3.2.7 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) to carry out Bayesian phylogenetic inference using a GTR+G+I model. This comprised two independent Markov chain Monte Carlo (MCMC) runs, with four chains per run, sampling every 1000 generations. **As the MCMC algorithm converges on the data over time, with the time it spends on a given tree equivalent to the Bayesian posterior probability (Huelsenbeck et al. 2001),** we discarded the first 25% of each MCMC run as a “burn-in” (Mau et al. 1999). **It is also important to ascertain that the MCMC algorithm has “converged” on a good approximation of the data and not merely a local maximum (Besag and Green 1993).** We assessed convergence in TRACER version 1.7.1 (Rambaut et al. 2018), deeming it acceptable once average standard deviation in split frequencies (ASDSF) reached 0.01 and Effective Sample Size (ESS) of model parameters reached 200 (O’Connell et al. 2019d). Both of these requirements had been met by the time our MCMC algorithm reached 4 million generations. We exported a 50% majority rule consensus tree from MrBayes and imported both

this and the ML tree into the R package “ggtree” (Yu et al. 2016). We produced a combined, simplified tree by omitting the outgroup and collapsing each major clade into a single branch, labelling the nodes with both ML bootstrap scores and Bayesian probabilities (Figure 2). We also made subtrees from the Bayesian tree, to display all haplotypes from *Monarcha* (Figure 3c) and *Hypothymis* (Figure 4c). Full versions of the Bayesian and ML trees, including all outgroup taxa, are provided in the Supplementary Material (Figures S3 and S4).

R code used in analyses

R Script

```
graphics.off()
rm(list=ls()) #WIPE

# -----
# PHYLOGENETICS
# -----

if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")

BiocManager::install("ggtree")
BiocManager::install("treeio")

library(treeio)
library(ggtree)
library(tidyverse)

#load ML consensus tree exported from IQTree
iqtreefile <- "D:/PhD/Monarchs/Monarchs_ML/IQTree/Monarch_Haplotypes_2021-06-09.fas.contree" #change path as
appropriate

iqtree1 <- read.newick (iqtreefile, node.label="support") #read the IQ Tree
iqtree2 = tree_subset (iqtree1, "MOCA_H01", levels_back=2) #subset tree to remove outgroup
iqtree3 <- ggtree (iqtree2) + geom_treescale() + geom_nodelab(aes(label = support)) #add labels and bootstraps
iqtree4 <- collapse(iqtree3, MRCA(iqtree3, "MOCI_W17", "MOCI_W11")) #collapsing Wakatobi ISMON
iqtree5 <- collapse(iqtree4, MRCA(iqtree4, "MOCI_M21", "MOCI_M18")) #collapsing Menui ISMON
```

```

iqtree6 <- collapse(iqtree5, MRCA(iqtree5, "MOCI_B02", "MOCI_B04")) #collapsing Djaul, Nusalaman, Sulei ISMON
iqtree7 <- collapse(iqtree6, MRCA(iqtree6, "HYPU_H05", "HYPU_H02")) #collapsing PBMON
iqtree8 <- collapse(iqtree7, MRCA(iqtree7, "HYAZ_H01", "HYAZ_H02")) #collapsing Black-naped Monarch

iqtree8

#load Bayesian consensus tree exported from MrBayes using sumt
MrBayesfile <- "D:/PhD/Monarchs/Monarchs_Bayesian/Monarch_Haplotypes_2021-06-09.fas.nex.con.tre" #change path as
appropriate

bayestree1 <- read.mrbayes (MrBayesfile)
bayestree2 = tree_subset (bayestree1, "MOCA_H01", levels_back=2) #subset tree so you only see one branch for
outgroup
bayestree2@data[["rprob"]] <- round(as.numeric(bayestree2@data[["prob"]]), digits=2) #rounding the Bayesian
probabilities to match the ML bootstraps
bayestree3 <- ggtree (bayestree2) + geom_treescale() + geom_nodelab(aes(label = rprob)) #add labels and bayesian
probability
bayestree4 <- collapse(bayestree3, MRCA(bayestree3, "MOCI_W17", "MOCI_W11")) #collapsing Wakatobi ISMON
bayestree5 <- collapse(bayestree4, MRCA(bayestree4, "MOCI_M21", "MOCI_M18")) #collapsing Menui ISMON
bayestree6 <- collapse(bayestree5, MRCA(bayestree5, "MOCI_B02", "MOCI_B04")) #collapsing Djaul, Nusalaman, Sulei
ISMON
bayestree7 <- collapse(bayestree6, MRCA(bayestree6, "HYPU_H05", "HYPU_H02")) #collapsing PBMON
bayestree8 <- collapse(bayestree7, MRCA(bayestree7, "HYAZ_H01", "HYAZ_H02")) #collapsing Black-naped Monarch

bayestree8

#making a Bayesian tree focused on Monarcha
monarchabayesian = tree_subset(bayestree1, "MOCA_H01", levels_back=1)
monarchabayesian@data[["rprob"]] <- round(as.numeric(monarchabayesian@data[["prob"]]), digits=2) #rounding

ggtree(monarchabayesian) + geom_tiplab() + geom_treescale(x=0,y=10) +
  geom_nodelab(aes(label = rprob), nudge_x = -0.0007, nudge_y = 0.25, size=3, colour = "black")

svg(filename="ISMONBayesianTree.svg",
  pointsize=14,
  width=25,
  height=10, bg="transparent")

```

```
ggtree(monarchabayesian, size=2, bg="transparent") + geom_tiplab() + geom_treescale(x=0,y=10, fontsize=8,
linesize=1) +
  geom_nodelab(aes(label = rprob), nudge_x = -0.0007, nudge_y = 0.37, size=9, colour = "black")
dev.off()
```

```
#making a Bayesian tree focused on Hypothymis
```

```
hypothymisbayesian = tree_subset(bayestree1, "HYAZ_H01", levels_back=2)
```

```
hypothymisbayesian@data[["rprob"]] <- round(as.numeric(hypothymisbayesian@data[["prob"]]), digits=2) #rounding
ggtree(hypothymisbayesian) + geom_tiplab() + geom_treescale(x=0,y=10) +
  geom_nodelab(aes(label = rprob), nudge_x = -0.0007, nudge_y = 0.25, size=3, colour = "black")
```

```
svg(filename="PBMONBayesianTree.svg",
  pointsize=14,
  width=25,
  height=10, bg="transparent")
ggtree(hypothymisbayesian, size=2, bg="transparent") + geom_tiplab() + geom_treescale(x=0,y=10, fontsize=7.5,
linesize=1) +
  geom_nodelab(aes(label = rprob), nudge_x = -0.0008, nudge_y = 0.37, size=7, colour = "black")
dev.off()
```

```
#exporting the full Bayesian and ML trees for the supplementaries
```

```
iqfull <- iqtreet1
```

```
svg(filename="MonarchMLTree_2021-06-10.svg",
  pointsize=10,
  width=25,
  height=10, bg="transparent")
ggtree(iqfull, size=1, bg="transparent") + geom_tiplab() + geom_treescale(x=0,y=10, fontsize=7.5, linesize=1) +
  geom_nodelab(aes(label = support), size=4, colour = "black")
dev.off()
```

```
bayesfull <- bayestree1
```

```
bayesfull@data[["rprob"]] <- round(as.numeric(bayesfull@data[["prob"]]), digits=2) #rounding
```

```

svg(filename="MonarchBayesianTree_2021-06-10.svg",
     pointsize=10,
     width=25,
     height=10, bg="transparent")
ggtree(bayesfull, size=1, bg="transparent") + geom_tiplab() + geom_treescale(x=0,y=10, fontsize=7.5, linesize=1) +
  geom_nodelab(aes(label = rprob), size=4, colour = "black")
dev.off()

# -----
# MORPHOMETRICS
# -----

ISMNMorph <- read.csv("SupplementaryFile2_Morphology.csv ") #change path as appropriate

adults <- ISMNMorph[which(ISMNMorph$age == "FG"),]

adults$wingspan <- ((adults$wing * 1.91) + 0.06) #calculate wingspan from wing length following Garrard et al 2012
and O'Connell et al 2019
adults$dispersal <- (adults$wingspan)^3 / adults$wt

t.test(adults$wingspan ~ adults$zone) #Menui population has much higher dispersal than Wakatobi

# -----
# BIOGEOGRAPHY
# -----

library("ggplot2")
library("ggrepel")

dist.data <- read.csv("SupplementaryFile3_Biogeography.csv ") #change path as appropriate

colnames (dist.data) <- c("island", "neighbour", "region", "heightm", "areakm2", "neighbourdistance", "meanp",
"dispersal")
rownames (dist.data) <- c("M", "W", "Kal", "T", "B", "H", "R", "L", "N", "D", "S", "Kar")

dist.data$log2distance <- log2 (dist.data$neighbourdistance)

```

```

wdata <- dist.data [which(dist.data$region == "Wallacea"),] #only the Wallacean birds have morphometrics

#MODEL A
# "Model A tests whether adjacent allopatric populations are more or less likely to diverge based on home island
size,
# with an expectation of a positive correlation between divergence and island area." Linck et al.

modelA <- lm (meanp ~ areakm2, data = dist.data)
summary(modelA)

#MODEL B
# "Model B tests whether adjacent allopatric populations are more or less likely to diverge based on home island
elevation,
# with an expectation of a positive correlation between divergence and maximum elevation." Linck et al.

modelB <- lm (meanp ~ heightm, data = dist.data)
summary(modelB)

#MODEL C (natural and log)
#"Model C tests whether the level of divergence between adjacent allopatric populations is related to the distance
between populations." Linck et al.
#Only significant if you log the distance or limit it to Wallacea

semilogmodelC <- lm (meanp ~ log2distance, data = dist.data)
summary(semilogmodelC)

#"Thus, models A and B test for reduced dispersal in supertramp populations on larger, less disturbance-prone
islands,
# while model C tests a classic isolation by distance hypothesis." - Linck et al

#MODEL W
#What is the relationship between genetic distance from nearest neighbour and dispersal ability?

modelW <- lm (meanp ~ dispersal, data = wdata)
summary(modelW)

```

```

#MODEL X
#What is the relationship between distance (km) (isolation) from nearest neighbour and dispersal ability?

modelX <- lm (neighbourdistance ~ dispersal, data = wdata)
summary(modelX)

#MODEL Y
#What is the relationship between island size and dispersal ability?

modelY <- lm (dispersal ~ areakm2, data = wdata)
summary(modelY)

#MODEL Z
#What is the relationship between island height (permanence) and dispersal ability?

modelZ <- lm (dispersal ~ heightm, data = wdata)
summary(modelZ)

#making better plots
coef(lm(meanp ~ areakm2, data = dist.data)) #need these for the abline of model A
coef(lm(meanp ~ heightm, data = dist.data)) #need these for the abline of model B
coef(lm(meanp ~ log2distance, data = dist.data)) #need these for the abline of model C

#switching to ggplot2 so i can use the ggrepel function to make legible labels

svg(filename="D:/PhD/Monarchs/Monarch_Distances/Monarch_Biogeography/ModelA.svg",      #change path as appropriate
    width=5,
    height=5, bg="white")
ggplot(data=dist.data, aes(areakm2, meanp, label = rownames(dist.data))) +
  geom_point(color = "black") +
  theme_classic(base_size = 12) +

```

```

geom_text_repel() +
  labs(x = bquote('Area of island'~(km^2)), y = "mean genetic divergence from nearest population") +
  geom_abline(intercept=2.667490e-04, slope=4.198715e-05 ) #coefficients calculated above
dev.off()

svg(filename="D:/PhD/Monarchs/Monarch_Distances/Monarch_Biogeography/ModelB.svg", #change path as appropriate
  width=5,
  height=5, bg="white")
ggplot(data=dist.data, aes(heightm, meanp, label = rownames(dist.data))) +
  geom_point(color = "black") +
  theme_classic(base_size = 12) +
  geom_text_repel() +
  xlab("Elevation of island (m)") +
  ylab(" ") +
  geom_abline(intercept=5.116383e-04, slope=1.392648e-05 ) #coefficients calculated above
dev.off()

svg(filename="D:/PhD/Monarchs/Monarch_Distances/Monarch_Biogeography/ModelC.svg", #change path as appropriate
  width=5,
  height=5, bg="white")
ggplot(data=dist.data, aes(log2distance, meanp, label = rownames(dist.data))) +
  geom_point(color = "black") +
  theme_classic(base_size = 12) +
  geom_text_repel() +
  xlab ("Log 2 (Kilometres from nearest population)") +
  ylab(" ") +
  geom_abline(intercept=-0.0009199186, slope= 0.00100476126 ) #coefficients calculated above
dev.off()

coef(lm(meanp ~ dispersal, data = wdata)) #need these for the abline of model W
coef(lm(neighbourdistance ~ dispersal, data = wdata)) #need these for the abline of model X
coef(lm(dispersal ~ areakm2, data = wdata)) #need these for the abline of model Y
coef(lm(dispersal ~ heightm, data = wdata)) #need these for the abline of model Z

svg(filename="D:/PhD/Monarchs/Monarch_Distances/Monarch_Biogeography/ModelW.svg", #change path as appropriate
  width=5,
  height=5, bg="white")
ggplot(data=wdata, aes(dispersal, meanp, label = rownames(wdata))) +

```

```

geom_point(color = "black") +
theme_classic(base_size = 12) +
geom_text_repel() +
xlab ("Dispersal Index (wingspan^3 / mass)") +
ylab("mean genetic divergence from nearest population") +
geom_abline(intercept=-1.924751e-02, slope=1.076123e-07 ) #coefficients calculated above
dev.off()

svg(filename="D:/PhD/Monarchs/Monarch_Distances/Monarch_Biogeography/ModelX.svg",      #change path as appropriate
width=5,
height=5, bg="white")
ggplot(data=wdata, aes(dispersal, neighbourdistance, label = rownames(wdata))) +
geom_point(color = "black") +
theme_classic(base_size = 12) +
geom_text_repel() +
xlab ("Dispersal Index (wingspan^3 / mass)") +
ylab("Kilometres from nearest population") +
geom_abline(intercept=-6.012130e+02, slope=3.145673e-03) #coefficients calculated above
dev.off()

svg(filename="D:/PhD/Monarchs/Monarch_Distances/Monarch_Biogeography/ModelY.svg",      #change path as appropriate
width=5,
height=5, bg="white")
ggplot(data=wdata, aes(areakm2, dispersal, label = rownames(wdata))) +
geom_point(color = "black") +
theme_classic(base_size = 12) +
geom_text_repel() +
xlab ("Island Area (km^2)") +
ylab("Dispersal Index (wingspan^3 / mass)") +
geom_abline(intercept=202916.77305, slope=-15.23496) #coefficients calculated above
dev.off()

svg(filename="D:/PhD/Monarchs/Monarch_Distances/Monarch_Biogeography/ModelZ.svg",      #change path as appropriate
width=5,
height=5, bg="white")
ggplot(data=wdata, aes(heightm, dispersal, label = rownames(wdata))) +
geom_point(color = "black") +
theme_classic(base_size = 12) +
geom_text_repel() +

```

```
xlab ("Island Height (m)") +
ylab("Dispersal Index (wingspan^3 / mass)") +
geom_abline(intercept=208635.96745, slope=-44.28923) #coefficients calculated above
dev.off()
```

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